Tumor-targeted Delivery of Paclitaxel using Low Density Lipoprotein-mimetic Solid Lipid Nanoparticles

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ABSTRACT SUMMARY
This study represents paclitaxel-containing lipid-based solid lipid nanoparticles (SLNs) with polyethylene glycol (PEG)-modified surfaces and tumor-targeting ligand. The prepared paclitaxel-containing tumor-targeting SLNs had a mono-dispersed size distribution with a neutralized surface charge. Paclitaxel loaded efficiently into these SLNs. In pre-clinical human cancer xenograft mouse model studies, these SLNs had superior anti-tumor activity to in-class nano-particular therapeutics in clinical use, and yielded long-term complete responses.

INTRODUCTION
Many nanoparticle-based approaches to cancer therapy have failed, due to their inherent toxicity, limited efficacy and stability.1,2 To resolve these engineering hurdles is an important step towards the successful development of novel nano-therapeutics.
In this study, we developed anti-cancer therapeutic lipid nanoparticles, which mimic LDL (low-density lipoprotein). This therapeutic nanoparticle contained water-insoluble anti-cancer drug, paclitaxel, in the core and have tumor-targeting ligand, cetuximab, covalently conjugated on the polyethylene glycol (PEG)-modified surface. Not only did we describe the engineering aspects of manufacturing these novel nanoparticles in detail, but we also validated their in vivo functionality through a well-controlled pre-clinical animal model studies with other clinically-approved in-class nanoparticles.

EXPERIMENTAL METHODS
Paclitaxel-containing SLNs (tSLNs) for therapeutics were manufactured using a modified solvent-emulsification method.3 Targeting moiety (cetuximab) was conjugated to the tSLNs using the hetero-bifunctional cross-linking agent, NHS-PEG-maleimide. Briefly, NHS-PEG-maleimide (2.7 mg) and NHS-PEG-methoxy (6.3 mg) were dissolved in deionized water at a concentration of 1 mg/ml and added to 1 ml of tSLN solution (5 mg/ml) with vigorous rotation for 12 hours at 4 ºC to covalently link between NHS groups on the PEG derivatives and primary amine groups on the tSLN surface. Thiolated cetuximab pretreated with Traut’s reagent, 2-iminothiolane, was then conjugated to the PEGylated tSLNs (PtSLNs). Thiolated cetuximab (1.1 mg) was incubated with 1 ml of PtSLN (5 mg/ml) at 4 ºC overnight. Thioether bond formation between the PtSLNs and thiolated cetuximab yielded cetuximab-conjugated PtSLNs (cetuxi-PtSLNs). Prepared SLN formulations were observed by AFM (XE-100, Park system, Korea) and Zetasizer nanoseries nano-ZS (Malvern Instruments LTD., Malvern, UK).
We also studied the anti-tumor efficacy of cetuxi-PtSLNs in comparison with the conventionally used taxane-delivering formulations, Taxol® and Genexol®-PM. Female SPF BALB/C-nu mice bearing lung cancer cell lines, H1975 cells in the right flank and H1650 cells in the left flank, were randomly divided into three groups (n=6 mice/group) and cetuxi-PtSLNs at their effective tolerable dose, eTD (22 mg paclitaxel/kg) and Taxol® and Genexol®-PM at their MTD (Taxol® : 20 mg paclitaxel/kg, Genexol®-PM : 60 mg paclitaxel/kg) were administered intravenously once a week for 3 weeks. Tumor size was monitored with calipers once a week for 11 weeks and tumor volume was calculated.

RESULTS AND DISCUSSION
The prepared cetuxi-PtSLNs (Figure 1) had a mono-dispersed size distribution (160.4 ± 0.9 nm) and neutralized surface charge value of - 1.3 ± 1.0 mV (Figure 2).
Figure 1. Schematic illustration of tumor-targeting PtSLNs (cetuxi-PtSLNs). Shown right and below are the synthetic steps used to prepare tumor-targeting PtSLNs. Inserted image was measured using AFM to determine the physical appearance of the nanoparticles.

Figure 2. (A) Hydrodynamic diameters and (B) ζ-potential values of SLN formulations in deionized water. Data presented are mean ± SD (n=3 independent measurements, ** p < 0.01; t-test).

Paclitaxel loaded efficiently into these SLNs (11 ± 0.8% w/w) as shown in table 1. The total paclitaxel loading amount in water (550 ± 40 μg/ml) at 5.0 mg/ml of SLN formulations was significantly higher than the solubility of paclitaxel in water (<1 μg/ml), suggesting that the improved solubilization of paclitaxel was mediated primarily by molecular dissolution in the hydrophobic core of the SLNs.

Table 1. The efficiency of paclitaxel encapsulation into the cetuxi-PtSLNs (determined by HPLC)

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<th>paclitaxel loading efficiency (%)</th>
<th>paclitaxel loading content (%, w/w)</th>
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<td>69 ± 5.3</td>
<td>11 ± 0.8</td>
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In pre-clinical human cancer xenograft mouse model studies, the targeted-PtSLNs exhibited superior anti-tumor activity to in-class nanoparticulate therapeutics in clinical use, and yielded long-term complete responses (Figure 3).

Figure 3. Comparison with in-class paclitaxel-based clinical therapeutics. (A-B) Tumor growth curves of nude mice bearing (A) H1975 xenograft and (B) H1650 xenograft were recorded after intravenous administration of cetuxi-PtSLN, Taxol® (n=6 for each group). (C) In vivo optical images of H1975 (right) and H1650 (left) tumor bearing mice on days 17 (high row), 38 (middle row) and 63 (low row) after intravenous administration of cetuxi-PtSLN, Taxol®, or Genexol®-PM. Black arrows indicate the time point of intravenous administrations of the therapeutic, and red arrows indicate tumor sites. All data are shown as mean ± SD.

CONCLUSION
Because of their excellent nano-scale reservoir structures for efficient encapsulation of therapeutic, high tumor targeting ability, and bio-safety, tumor-targeting SLN formulations can potentially be used as delivery vehicles for water-insoluble anti-cancer drugs, facilitating effective cancer therapy while reducing non-specific side effects.

REFERENCES

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